REMARKS

The specification has been objected to, claims 15-16 have been rejected under 35 U.S.C. § 112, and claims 12-16 have been rejected under 35 U.S.C. §103(a).

An new Abstract has been provided to overcome the objection to the specification in Item 4 of the Office Action.

Claim 15 has proper antecedent basis. Claim 16 has been amended to overcome the antecedent basis rejection in Item 5 of the Office Action.

New claims 21-23 have been added to further define the invention of claim 12, and have a basis at paragraph [0015].

Claims 12-16 have been rejected under 35 U.S.C. § 103(a) in view of U.S. Patent No. 6,087,032 to Yoshitake *et al.* ("Yoshitake") and US 2002/0096279 to Kinsley Jr. ("Kinsley") and U.S. Patent No. 5,207,826 to Westland *et al.* ("Westland").

Yoshitake was cited as disclosing a fuel cell comprising an electrolyte membrane, a fuel electrode and an air electrode. The Office Action concedes that Yoshitake does not disclose specific anode or cathode material. It should be further noted that Yoshitake does not disclose the use of bacterial cellulose in an electrolyte membrane.

Kinsley was cited as teaching the use of bacterial cellulose for fuel cell electrode purposes. However, upon further review it can be seen that Kinsley does not disclose an electrode comprising bacterial cellulose. Attention is first directed to paragraph [0023] where it states that metal fibers may be dispersed in a slurry composed only of water and a high surface area material like bacterial cellulose. Then, at paragraph [0026] it states that "once the metal fibers have been dispersed in the aqueous

dispensing fluid, the dispensing fluid is then applied to a screen as is conventional in papermaking process," and "the aqueous dispensing fluid is then removed in order to form the metal fiber sheet." Thereafter, at paragraph [0031] it states that

> The final step is a sintering step which can be conducted at optimum temperatures in an inert or reducing atmosphere. The sintering step introduces a strength to the metal fiber paper, <u>as well as burns off the various organics contained in the metal fiber paper. The sintering step generally involves heating the paper at a temperature of from 1500-1200°F. for a time necessary to burn off the organics.</u> (Underlining added.)

At paragraph [0037], it notes that the resulting metal fiber sheet can be used as a battery electrode.

These paragraphs from Kinsley clearly show that the electrode described in Kinsley does not contain bacterial cellulose as recited claim 12. The bacterial cellulose is used in Kinsley merely as a dispersing agent, and the sintering step in Kinsley will necessarily burn off the cellulose. Hawley's Condensed Chemical Dictionary lists the ignition point of cellulose as approximately 450°F and therefore, given the sintering temperature listed in Kinsley (1200-1500°F), no cellulose will remain in the resulting electrode. Thus, it is respectfully submitted that Kinsley fails to teach an electrode comprising bacterial cellulose.

Westland was cited in the Office Action as teaching that bacterial cellulose can be used as membranes and/or specialty components for fuel cells and/or materials having special electronic properties. In particular, the Office Action makes reference to column 2, lines 56-69 of Westland where Westland references the teachings of WO 89/12107 when describing various uses of bacterial cellulose.

Applicant was not aware of WO 89/12107 until Westland was cited in the Office Action and therefore, a copy of WO 89/12107 has now been reviewed. The Applicant encloses a Form PTO-1449 with this document listed and respectfully requests that WO 89/12107 be considered by the Examiner, be made of record in the present application and that an initialed copy of the Form PTO-1449 be returned in accordance with MPEP § 609.

Upon review of WO 89/12107, Westland appears to be making reference to page 3, line 28 to page 4, line 2 of WO 89/12107 where some uses of bacterial cellulose are broadly described. However, the mention of "membranes" in WO 89/12107 says nothing about fuel cell components. It is describing filtration and separation media such as membranes for water purification (see page 3, lines 28-31 of WO 89/12107).

WO 89/12107 does mention "a specialty carrier, such as for battery fluid and fuel cells" at page 3, lines 32-33. However, "a specialty carrier" could mean anything and in fact, page 17, lines 33-36 of WO 89/12107 describe the specific use of bacterial cellulose contemplated in WO 89/12107 as "gels useful in fuel cell and battery structure articles, where the electrolyte comprises the liquid phase of the microbial cellulose gel." Thus, the "specialty carrier, such as for battery fluid and fuel cells" described in WO 89/12107 and made referenced to by Westland is an <u>electrolyte membrane</u> where the electrolyte comprises the liquid phase of the microbial cellulose gel. Nothing in WO 89/12107 or Westland describes the use of bacterial cellulose in a fuel cell anode or a fuel cell cathode as specifically recited in claim 12.

WO 89/12107 also mentions "materials having special electronic effects produced by coating the individual microbial-produced, cellulose, microfibrils with

appropriate components, such as metals by vapor deposition or epitaxial growth" at page 3, line 34 to page 4, line 2. However, what is being referred to is the production of conducting, semiconducting or superconducting dry ribbons or thin films of metal coated cellulose (see page 11, lines 25-33 and page 14, lines 13-19 of WO 89/12107). There is no mention of the use of bacterial cellulose in a fuel cell anode or a fuel cell cathode as specifically recited in claim 12.

There are further fundamental differences between the present invention and the teachings of Yoshitake, Kinsley and Westland. The method used for formation of a membrane or sheet of cellulose in these patents is the same as that used for casting sheets of paper in the paper and pulp industries, that is, a suspension or slurry of mechanically disrupted cellulose fibers that are obtained from a natural source, either plant-derived or bacterial, is laid or rolled out and dried. The method of the present application uses the sponge-like bacterial cellulose pellicule without disruption of its natural structure and microarchitecture. The bacterial cellulose described by Westland is produced in agitated culture from a strain that produces cellulose in the form of lose pellets or pieces, instead of a contiguous, sponge-like gel pellicule, such as those employed by the present inventors. These hydrated pellicules can be treated with different chemicals to form metallized electrode materials or proton-conductive materials, and then assembled by serially drying one onto the other. A stable membrane sandwich is formed by hydrogen bonds between the hydroxyl groups of the cellulose chains.

The method used Yoshitake, Kinsley and Westland for incorporation of metal particles employ one of two processes: (1) preformed metal particles that are then

entrapped in the cellulose matrix during the sheet casting process; or (2) metal particles that deposited or epitaxially grown on the surfaces of the cellulose by vaporization of the metal or metals in question in special chambers. In our patent application, the preferred embodiment is the deposition of metal particles from the corresponding metal salts in aqueous solution that are infused into the natural cellulose structure. Particle formation is then initiated by reduction of hexachloropalladate by the reducing ends of the cellulose chains. Cellulose is a polymer of glucose, and the reducing ends have free aldehyde groups as does glucose in solution. An alternative method is the infusion of another chemical that can reduce the metal salts inside the cellulose matrix. The inventors have tunneling electron microscopy images of the palladium particles formed by this method that show size (5-20 nm) and crystallinity of the palladium particles.

In summary, (1) Yoshitake does not disclose the use of bacterial cellulose in an electrolyte membrane or a fuel cell anode or a fuel cell cathode as recited in claim 12; (2) the electrode described in Kinsley does not contain bacterial cellulose as recited claim 12; and (3) Westland, and WO 89/12107 which is referenced in Westland, merely describe a "specialty carrier, such as for battery fluid and fuel cells" which turns out to be an electrolyte membrane where the electrolyte comprises the liquid phase of the microbial cellulose gel and therefore, nothing in WO 89/12107 or Westland describes the use of bacterial cellulose in a fuel cell anode or a fuel cell cathode as specifically recited in claim 12. Thus, all of the limitations of claim 12 (and claims 13-16 and 21-23 that depend thereon) are not taught in any combination of Yoshitake, Kinsley, Westland and WO 89/12107.

Conclusion

It is submitted that the entire application has been placed in condition for allowance. Favorable reconsideration is respectfully requested. A fee sheet is attached for the extra three claims. No other fees are believed to be needed for this amendment. If additional fees are needed, please charge them to Deposit Account 17-0055.

Respectfully submitted,

Barbara R. Evans *et al.*

Dated: February 6, 2004

By: Richard T. Roche

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5481969v2

ABSTRACT OF THE DISCLOSURE

The employment of metallized bacterial cellulose in the construction of fuel cells and other electronic devices is disclosed. The fuel cell includes an electrolyte membrane comprising a membrane support structure comprising bacterial cellulose, an anode disposed on one side of the electrolyte membrane, and a cathode disposed on an opposite side of the electrolyte membrane. At least one of the anode and the cathode comprises an electrode support structure comprising bacterial cellulose, and a catalyst disposed in or on the electrode support structure.

cellular plastic. A thermosetting or thermoplastic foam composed of cellular cores with integral skins having high strength and stiffness. The cells result from the action of a blowing agent, either at room temperature or during heat treatment of the plastic mixture. The resulting product may be either flexible or rigid, the latter being machinable. The foaming action in some cases may occur *in situ* (foamed-in-place plastics). Cellular plastics are combustible. For details, see foam, plastic.

Use: (Flexible) Furniture, automobile interiors, matresses, etc., where softness and resiliency are desired. (Rigid) Insulating material, boat building and similar light construction, salvage of waterlogged ships.

See also foam, plastic; rubber sponge. For further information, refer to Cellular Plastics Division, Society of the Plastics Industry, 355 Lexington Ave., New York, NY 10017.

cellulase. An enzyme complex produced by the fungi *Aspergillus niger* and *Trichoderma viride* which is capable of decomposing cellulosic polysaccharides into smaller fragments, primarily glucose. It has been used as a digestive aid in medicine and in the brewing industry. Research has been devoted to experimental application of cellulase to disposal of cellulosic solid wastes. The resulting glucose can be fermented to ethanol, used to grow yeast for animal-feed proteins, or used as a chemical feedstock.

Note: Cellulase derived from the thermophilic soil fungus *Thielatia terrestris* functions at a much higher temperature than other types and is thus much more effective in decomposing cellulose. This indicates its possible use in conversion of biomass to energy. Commercial development of this product is expected.

See also biomass.

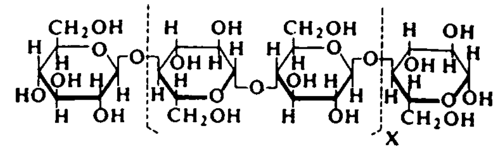
"Celluloid" [*Hoechst Celanese*]. CAS: 8050-88-2. TM for a plastic consisting essentially of a solid solution of cellulose nitrate and camphor or other plasticizer plus a flame retardant such as ammonium phosphate to minimize flammability; available in sheets, rods, tubes, films. Also called pyroxylin.

Hazard: Flammable, dangerous fire risk.

See also nitrocellulose.

cellulose. CAS: 9004-34-6. $(C_6H_{10}O_5)_n$.

A natural carbohydrate high polymer (polysaccharide) consisting of anhydroglucose units joined by an oxygen linkage to form long molecular chains that are essentially linear. It can be hydrolyzed to glucose. The degree of polymerization is from 1000 for wood pulp to 3500 for cotton fiber, giving a molecular weight from 160,000 to 560,000.



Cellulose is a colorless solid, d approximately 1.50, insoluble in water and organic solvents. It will swell in sodium hydroxide solution and is soluble in Schweitzer's reagent. It is the fundamental constituent of all vegetable tissues (wood, grass, cotton, etc) and is the most abundant organic material in the world. Cotton fibers are almost pure cellulose; wood contains approximately 50%.

The physical structure of cellulose is unusual in that it is not a single crystal, but consists of crystalline areas imbedded in amorphous areas. Chemical reagents penetrate the latter more easily than the former. Cellulose is virtually odorless and tasteless and is combustible, with an ignition point of approximately 450F. In some forms it is flammable. For example, railroad shipping regulations require a "flammable" label on such items as "burnt fiber," "burnt cotton," "wet waste paper," and "wet textiles." Fires have been known to occur in warehouses in which telephone books were stored. These were undoubtedly due to heat buildup in the paper caused by microbial activity and self-sustaining oxidation.

See also flammable material.

The most important uses of cellulose are bulk woods of many kinds; paper, most of which is made from wood pulp; cotton products (clothing, sheeting, industrial fabrics); packaging, ranging from wooden barrels to candy pats; and as a source of ethanol (enzymatic hydrolysis) and methanol (destructive distillation of wood). Specialized uses include nonwoven fabrics, medical equipment (artificial kidney), insulation and soundproofing, sausage casings, etc. Cellulose has approximately 60% of the energy content of bituminous coal; its use as a fuel has increased, especially in rural locations.

See also biomass.

There are many chemical modifications of cellulose, including its esters (cellulose acetate), ethers (methylcellulose), the nitrated product (nitrocellulose), and rayon and cellophane (from cellulose xanthate). Thus, it is the basis of many plastics, fibers, coatings, lacquers, explosives, and emulsion stabilizers. Alkali cellulose is an intermediate made by the action of sodium hydroxide solution on cellulose and is used for making cellulose ethers and viscose.

See also cellulose, modified.

Cellulose exists in three forms–α, β, and γ. α-cellulose has the highest degree of polymerization (DP), and is the chief constituent of paper pulp. It is insoluble in strong sodium hydroxide

OIPE JC109 FEB 10 2004 PATENT & TRADEMARK OFFICE

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 54.00

Complete if Known	
Application Number	10/017,202
Filing Date	12-14-2001
First Named Inventor	Barbara R. Evans
Examiner Name	Raymond Alejandro
Art Unit	1745
Attorney Docket No.	920976.90199

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

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Deposit Account Number: 17-0055

Deposit Account Name: Quarles & Brady LLP

The Director is authorized to: *(check all that apply)*

☑ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

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to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims	23	-20* =	3	x	18	=	54.00
Independent Claims	5	-5** =	0	x		=	0.00
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 54.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) __________

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0.00

SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	Richard T. Roche	*Registration No. (Attorney/Agent)*	38,599	*Telephone*	414-277-5805
Signature	Richard T Roche			*Date*	2-6-2004

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	10/017,202
Filing Date	12/14/2001
First Named Inventor	Barbara R. Evans
Group Art Unit	1745
Examiner Name	Raymond Alejandro
Attorney Docket Number	920976.90199

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	Kind Code[2] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Office[3]	Number[4]	Kind Code[5] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO	89/12107	A1	Malcolm R. Brown	12-14-1989		

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] Unique citation designation number. [2] See attached Kinds of U.S. Patent Documents. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

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A1

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(71)(72) Applicant and Inventor: BROWN, R., Malcolm [US/US]; 305 Skyline Drive, Austin, TX 78746 (US).

(74) Agent: PAUL, Thomas, D.; Fulbright & Jaworski, 1301 McKinney, Houston, TX 77010 (US).

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Published
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(54) Title: MICROBIAL CELLULOSE AS A BUILDING BLOCK RESOURCE FOR SPECIALTY PRODUCTS AND PROCESSES THEREFOR

(57) Abstract

The production of articles from bacterial cellulose is disclosed. A novel process also is disclosed for manufacturing bacterial cellulose which, in turn, is useful for producing a variety of articles.

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MICROBIAL CELLULOSE AS A BUILDING BLOCK RESOURCE FOR SPECIALITY PRODUCTS AND PROCESSES THEREFOR

BACKGROUND OF THE INVENTION

This invention relates to a method of manufacturing for a multitude of new speciality products utilizing microbial cellulose. The present invention also relates to the products made according to this method. Ultrathin-superstrong transparent files and tissue growth medium article/compositions, especially self-supported and with nutrients incorporated therein, are outstanding examples of products according to the present invention. Speciality papers, transport media, chemical derivatives, etc., from microbial gels are also features of the invention described herein.

The prior art has generally recognized that gels formed of microbial-produced-cellulose microfibrils (pellicles) are essentially usable directly for certain end-use and product applications in which the cellulosic characteristic of microbial-produced cellulose

microfibrils is applied as a substitute for conventional cellulose. Copending United States Application No. 684, 844, filed December 21, 1984, is an illustration of an in situ utilization of the cellulose forming ability of certain microbes.

Examples of this substitution mode in the prior art include U. S. Patent 4,588,400 in which microbial produced cellulose microfibrils (MC) pads are used to retain medical fluids analogously to a cotton pad or a fabric holding liquids. Also, the present inventor's United States Patent No. 4,378,431 utilizes the cellulosic character of microbial-produced cellulose microfibrils to coat other fibers and fabrics to impart a bulk cellulosic characteristic to the surface thereof. Thereby, articles composed of such coated fibers have the feel, dyeability, printability, liquid sorbtion and other characteristics of cotton fabrics. European Application NO. 0228779 is an example of a process patent directed to a reticulated fibril configuration.

While the above-noted patents reflect quite recent developments in this area, further advances have been made. These advances have resulted in improved cellulose product and ultimately improved or completely new applications of same in end products.

SUMMARY OF THE INVENTION

The generic essence of the present invention is the discovery that improved microbial-produced, cellulose microfibrils (MC) can be produced and used as a much more versatile intermediate and starting material building block than previously realized, in order to form numerous new classes of unique derivative products. These products have properties and characteristics not obtainable from conventional large fiber cellulosics and are not contemplated by the prior MC art.

The process methodology for relating microbial cellulose properties with end-use products is also a feature of this invention.

Thus, the broad scope of the instant invention comprehends the processes for making such novel products, the products per se, as well as the processes for using them. In many instances the use for a particular product describes the utility for the unique processes and products of the invention, all of this being detailed more fully below as best known inventive embodiments.

DESCRIPTION OF PREFERRED EMBODIMENTS

Although many specific embodiments will be described herein, it will be apparent that part of the methodology of the invention is the discovery of improved methods of producing microbial cellulose from micro-organisms. The resulting cellulose has a large number or set of properties and processes which can be collected and compiled for transposing microbial-produced cellulose microfibrils into compositions, articles, and structures having nonobvious properties, not previously known for cellulosic compositions.

Microbial cellulose, as a substitute for conventional cellulose and for applications in which conventional cellulose was not previously used, finds a variety of uses. The following list is exemplary:

(A) nonwovens and films, including speciality papers, filtration and separation media, also including membranes that could be used for water purification, films on which inorganic films can be deposited and the like;

(B) a speciality carrier, such as for battery fluid and fuel cells;

(C) materials having special electronic effects produced by coating the individual microbial-produced,

cellulose, microfibrils with appropriate components, such as metals by vapor deposition or epitaxial growth;

(D) carriers for body-related materials, such as foods, cosmetics, skin/hair treating materials and internal drugs;

(E) mixing agents and viscosity modifiers per se or as chemically and physically modified, in applications such as surface coatings, particularly paints and fillers, plasters, glues, adhesives, grouts and caulks;

(F) new specialty fibers, such as carbonized versions, which can be used as polymer fillers and also noncarbonized fibers, especially when strength and biodegradeability are desired;

(G) light transmitting optical fibers;

(H) wavelength and other electromagnetic and radiation modifying materials;

(I) microfiber blends, especially with melt-blown and other polyolefin fibers and in admixture with many other different types of fiber to achieve special effects and biodegradeability;

(J) substrate media, especially self-supported forms for growing plant and animal tissue;

(K) foods, food substrates and good fiber substitutes;

(L) speciality laboratory uses such as for testing for cellulase activity and substrates for biological separations;

(M) other shapes such as specialty clothing, which is lint-free;

(N) special property-modifying process steps and modifications;

(O) synthetic leather and other texturized and special appearance surfaces;

(P) diet fiber substitutes, such as for psyllium fibers and in admixture with other dietary fibers, such as

wheat and oat (MC has attributes of soluble fiber because of its submicron dimensions);

(Q) blends with other fibers, such as cotton as a substitute for synthetics, such as polyesters and nylons, in woven fabrics and nonwoven articles; and

(R) moisture-absorbing soil-enhancing additives and conditioners.

The processes by which microbes produce microbial-produced-cellulose-microfibrils are well-known to the art. In general, the technique is described in the inventor's previously issued U. S. Patent 4,378,431, the disclosure of which is hereby incorporated by reference.

Any microbial strain capable of generating cellulose is generally usable for the processes, articles and compositions of the invention. These will be generically referred to as cellulose producing microbes (M). More specifically, those in the Acetobacter, Rhizobium, Agrobacterium and Pseudomonas genera, as described by the present inventor in his article in the J. Applied Science: Appl. Polymer Symp. (1983) 37, 33-78, the disclosure of which is hereby incorporated by reference, are preferred. The species Acetobacter xylinum is particularly preferred.

A novel discovery and part of the unique process covered by the present application is that improved cellulose results from the particular selection of microbe strains which are capable of reversing direction of travel during cellulose synthesis. Such a reversal results in a more dense and stronger cellulose. Particularly preferred is the NQ-5 strain of Acetobacter xylinum (American Type Culture Collection 53582). A more detailed explanation of this phenomenom is described in co-pending U.S. Application No. 023,336, filed March 9, 1986, and entitled, "Multi-Ribbon Microbial Cellulose", the disclosure of which is hereby incorporated by reference.

Growth of the cellulose is promoted by a culture medium which contains the microbes. The major constituent of the culture medium for Acetobacter is a soluble saccharide, particularly sugars, most particularly a hexose and especially glucose.

Suitable nutrients are well known to the art. One known as Schramm & Hestrin medium is especially preferred. It generally comprises about 20 g/l glucose, 5 g/l peptone, 5 g/l yeast extract, 2.7 g/l anhydrous diabasic sodium phosphate, and 1.15 g/l citric acid monohydrate. Corn steep liquor and molasses are practical and inexpensive sources of the hexose component preferred in the nutrients of the invention. Another satisfactory nutrient composition comprises about 8 volume percent vinegar, 5 volume percent ethanol and 4 weight percent malt extract. The pH is preferably adjusted to about 3 to 6, most preferably about 3.5 to 5.5. When it is desired to increase the amount of oxygen-containing components in the nutrient, additional alcohols and mixtures thereof can be included in the nutrient.

The ambient temperature for maximum effectiveness of microbial cellulose production is about 15 to 40, preferably about 20 to 30 degrees Centigrade. The total amount of time needed for acceptable cellulose production is generally from about 1 to 25 days. Techniques for improving microbe growth and increased cellulose production from each microbe are contemplated by this invention.

In copending, United States Application No. 684,844, filed December 21, 1984, and entitled "Production of Microbial Cellulose", the disclosure of which is incorporated herein by reference, on which the instant inventor is a coinventor, a comprehensive inventive scheme is disclosed for utilizing cellulose producing microbes for producing shaped cellulosic objects on or within a

template. Various chemical and physical modifications are disclosed to enhance and improve such shaped objects. Similarly, this inventor's U. S. Patent No. 4,378,431 utilizes an existing fibrous structure as a template/substrate for depositing a layer of cellulose in situ from cellulose producing microbes. This approach essentially utilizes the shape-forming ability of cellulose producing microbes to form shapes that could also have been formed from slurries of cotton/conventional cellulosic fibers, even though not so practical a process as that based on cellulose-producing microbe techniques.

The conventional product of cellulose-producing microbes is a mass of intertwined ribbons comprised of cellulosic microfibrils. These ribbons are generated at the oxygen-containing gas (air is operable)-nutrient interface. This mass is translucent, insoluble, but very hydrophilic and wettable and has great tensile strength. It appears to be a gel to sight and touch. It, as well as products made therefrom, have exceptionally high dry tensile strengths and dimensional stability.

One of the features of this invention is that the oxygen/liquid nutrient interface can be conveniently obtain by growing the microbes in an enclosed plastic film or bag. Each such container is a discrete reactor and can be designed to be of any size not exceeding the bursting strength of the container or its sealing means. It is preferably oblong, about 0.5 to 2, preferably about 0.75 to 1.5 feet in width and about 0.25 to 0.6, preferably about 0.3 to 0.5 feet in height. It should be at least about 0.5 feet in length.

Air space is provided above the liquid surface in the container. It is desirable to utilize a plastic film having the characteristic of a relatively high oxygen diffusion ability with low permeability to the liquid nutrient molecules. This practical, versatile reactor can

be designed for any location and has particular applicability in remote areas. If desired, the oxygen-containing environment within the reactor can be increased to improve oxygen availability to the microbes and, thereby, obtain a more efficient cellulose conversion.

In addition to the foregoing advantages, these reactors are desirable because the contamination problems ordinarily plaguing biotech processes can be easily controlled and eliminated. While these reactors have been described in connection with cellulose producing microbial processes, they are intended to be used in any bioprocess in which their usefulness can be enjoyed.

Another feature of the invention is the recognition that an improved cellulosic product is obtained by adding an agent to the nutrient bath which interferes with crystallization, but not polymerization, of the cellulose. Suggested agents include dextran having substituent groups such as akyl, alkyl carboxyl, alkylhydroxyl, sulfate, sulfonic acid or alkylphosphate. Particularly preferred is carboxymethyl-cellulose (CMC). This concept is described in more detail in United States Application No. 022,904 filed March 6, 1987, and entitled "Microbial Cellulose Modified During Synthesis", the disclosure of which is hereby incorporated by reference.

The generic concept of the present invention transposes the improved microbial cellulose into novel and nonobvious products, which utilize the special properties of microbial cellulose produced according to the present processes, which are not obtainable from other cellulosic sources or other microbial cellulose produced to date. One of the breakthrough, inventive concepts of the present invention is the realization that the unique properties of cellulose producing microbes can be collected, catalogued and innovatively harnessed to customize unique products. Certain final product properties are defined and those

corresponding properties are selected so that microbial cellulose is adopted and tailored to be utilized in a huge variety or processes, products and compositions having no counterpart in the prior art.

The cellulose microfibrils produced from microbes have submicron cross-sectional diameter dimensions of from 1.5nm (nanometers) [0.0015 micron] to 10nm (0.01 micron) This results in an enormous fiber surface area per cubic volume of fiber. Moreover, the submicron dimensioned cellulose fibrils produced by microbes having exceptionally high wet and dry tensile strengths. These microfibrils are especially noteworthy with respect to their remarkably high length to diameter ratio which can be in the order of as much as millions to one.

Dispersions of wet submicron fibers can be wet spun or pulled into larger fibers or yarns of filaments of exceptional high tensile strength and Youngs Modulus. This can be accomplished with the pure MC cellulose fibrils, as well as chemical, genetic and physical modifications thereof, both before and after the process of producing the larger materials from the submicron fibrils.

In addition, the fibers, filaments or yarns can be carbonized. They have exceptional strength because parallel molecular orientations can be obtained. Polyacrylonitrile (PAN) fibers are currently the choice of the art for maximum strength. Unmodified microbial cellulose can be carbonized to approximate these properties. Further, grafts of acrylonitrile can be made to microbial cellulose. That grafted product will be a composite of the preferred properties of microbial cellulose and PAN for a preferred starting material for fiber carbonization.

These PAN grafts have inordinately high water absorption capabilities. The Department of Agriculture

recently patented a PAN-starch graft or copolymer which purportedly will absorb up to 1,000 times its weight of water. Microbial cellulose-PAN (MC-PAN) has at least comparable absorption properties; and, because of its fibrous characteristic, it can be used in environments in which structural integrity, as well as wettability, is important. For instance, an irrigating hose formulated from MC-PAN would constantly drip water from a saturated state. The tissue growing aspects described later herein will be enhanced in some aspects by the used of MC-PAN.

Although a dried pellicle or film from cellulose producing microbes has some paper-like aspects, the invention goes beyond that primitive level and advances the state of the paper/non-woven and film art dramatically. The key is that microbial cellulose according to the present invention has greatly different physical characteristics than conventional cellulosic fibers. This factor is inventively utilized to select certain types of microbial cellulose-based articles, such as specialty papers, that especially benefit from those special microbial cellulose properties. One of these is speciality paper, particularly those that need to be free of inorganic acids to avoid degradation.

Such papers include formal documents exemplified by diplomas, treaties, certificates and the like. These will be superior in aging, bending-resistance, tear resistance and other strength factors. Some of the process embodiments described elsewhere in this application, such as glycerol and CMC, can be used to enhance these properties. This utility is described in more detail in United States Application No. 199,780, filed May 31, 1988, and entitled "Microbial Cellulose Composites and Structures from in situ Formation".

Thus, in those paper, film membrane and other related applications, where flexibility and bending

strength, particularly at low temperatures, are desired, the MC can be treated with glycerol to obtain a vast improvement in these already respectable capabilities of MC.

In particular, the assemblage of submicron range diameter microfibrils results in a paper that also has an outstanding ability to accept inks, dyes, toner and other color impressions resulting in images of far greater resolution than is possible with conventional cotton linters, rag or wood based papers.

Moreover, the high quality paper as described above is specially adapted to be coated with photographic emulsions to produce photographs of very fine grain and definition. This permits the elimination of resin coating which is ordinarily utilized to mask the roughness of conventional papers.

To much the same effect, the microbial cellulose substrate can be coated with magnetic media and media capable of deformation often by heat for optical reading purposes under laser light. In addition to the receptivity and adherability of magnetic and photographic coatings, the strength and dimensional stability of microbial cellulose, especially at temperature extremes, all contribute to a superior support material.

The multiplicity of submicrobial fibers in a dry-state article can be coated with appropriate substances, especially in thicknesses as thin as single molecular layers of various materials, such as, conductors, to make the entire article electrically conducting. In some instances the selection of deposition material is effected so as to achieve any of superconductive, ordinarily conductive or semiconductor properties. Furthermore, the dry ribbons and thin films of MC in either the coated or noncoated stage can be oriented and adjusted to achieve different absorption properties for light and electromagnetic radiation.

Microbial cellulose paper and three dimensional dry industrial applications articles are especially suitable for certain industrial applications. These uses include:

(a) as a base for a polymerizable monomer or resin impregnated for such uses as circuit boards, friction discs for transmission plates and any application where strength and superior impregnability factors are important. MC in dispersed fiber form can be used as a reinforcing agent in a wide variety of composite articles; and

(b) diaphragms to be vibrated for sound transmission in both set and dry environments, such as those in loudspeakers, earphones, telephone transmitters. These diaphragms may be metal-coated for enhanced properties.

Resin impregnated or coated MC films, paper, or other shape can be built up into three dimensional thermosetting resin articles, where they will have great extreme temperature stability and useability. One excellent unique application is to form the molded nose cone of rockets, where they will provide ablative barriers for heat-resistance to atmospheric friction. Also in submarine nose cones, they will provide the resistance to pressure and temperature extremes and be used to house sonar generating equipment without metal barriers.

Even more unusual is the ability of diaphragms, membranes and films of microbial cellulose to be used under water, not only for sonar devices, but for underwater sound applications yet to be developed because no membrane of this type has been hitherto available. In hot water, the dimensional stability of these materials is exceptionally useful.

The ability of microbial cellulose structures, such as membranes and films to retain their structural integrity while wet and under water is especially important in many membrane applications. For instance, in the human body, kidney and heart implant membranes will play useful roles, especially since extremely thin and small articles can be used because MC has such strength and stability even in ultrathin cross sectional forms. And outside the body, blood and other body fluids can be treated and filtered. Water purification, such as by reverse osmosis on both small scale personal as well as large scale industrial situations, is well-suited for MC membranes, etc. Other separations where small pores from submicron microbial fibers accompanied by unusual strength are important factors can be achieved by microbial cellulose in appropriate shapes and configurations.

Moreover, suitably shaped three-dimensional MC items can be used as surgical implants in both gel and dry forms. In the dry form, it provides a multiplicity of interstices in which body tissue can renew itself for good healing and bonding. It is likely that MC will not stimulate extreme body rejection mechanisms, but it can be impregnated with clyclosporin and the like to minimize rejection problems.

Also, the microbial cellulose paper can be employed in a transparent film, which is molded or cast to be used as negatives and color transparencies in the graphics and reproduction industries.

In addition to the high strength and modulus generally characterizing the products of this invention, special mention must be made of the exceptional dimensional stability of these products, both at extreme high and low temperatures and with respect to very thin planar film or membrane forms, as well as small cross-sectional three-dimensional shapes.

One exceptionally unusual discovery is that remarkably thin films can be cast from dispersions of microbial cellulose, particularly when treated with glycerol or CMC or both. These films have a thickness of less than about 0.1 micron, e.g. 80 nanometers or 0.08 microns. The unusually thin films have remarkable strength and dimensional stability. Before drying they can be stretched for orientation to further enhance strength and stability properties. Moreover, they and other film/membranes of the invention can be twisted into filaments/threads/fibers, also with outstanding strength/stability properties.

It has been further discovered that inorganic materials, such as metals can be vapor deposited or epitaxially grown in approximately monomolecular layers on the surface of these ultra thin planar/filamentous materials. Thus, conducting, semiconducting and superconducting materials can be formed in such ultra thin layers on these ultra thin microbial cellulosic structures.

Since microbial cellulose is capable of remaining flexible and stable at temperatures as low as those of liquid helium, laminar composites of microbial cellulose and films of superconductive materials offer considerable promise in providing flexible superconductor conduits and structures that are not possible to achieve with the brittle, almost-ceramic, new superconductors that have recently excited the scientific/industrial community.

The May 24, 1988 Wall Straat Journal reported that thin layers of the new thallium superconductor are adequate conductors of electricity for some commercial applications. It was demonstrated to conduct 110,000 amps per square centimeter. The layers are presently being deposited on silicon. Depositing on microbial cellulose substrates according to the instant invention, opens the door to an enormous potential for flexible electronic

components at extremely low temperatures and/or in exceptionally small housings.

Films of this microbial cellulose are also excellent for use as edible casings, such as those used for sausages and hot-dogs.

The properties delineated above also lend themselves to microbial cellulose papers that serve as excellent wall coverings. They can be easily texturized and provide the strength of vinyl wall coverings without any loss of breathability of the wall, thereby obviating unwanted vapor barriers in a room.

These properties of a microbial cellulose are also applicable to electrical insulating applications, especially when very high electrical power loads are involved. They are also equally applicable for thermal insulating environments. Any shape can be fabricated. Non-melting and cold-insensitive microbial cellulose structures are particularly suitable in extreme temperature environments.

These same insulating and breathing properties are vital to achieve special benefits for clothing to be used in specially harsh environments, such as the space program. In that connection, the extra-vehicular activity suit, as well as the ones worn on the space vehicle must be lint-free. That is a special property of microbial cellulose.

This type of clothing, including hand-gloves where suitable, must be pressure and puncture resistant, again a special property of microbial cellulose. Moreover, the gloves and clothing retain their flexibility at extremely low temperatures. They are also useful for civilian applications such as skiing and mountain climbing clothing.

All clothing items and insulators can be formed by in situ means, such as described in U.S. Application No. 684,844.

Special texturized and insulating effects can be obtained by freeze-drying a pellicle. The surface of the resulting product appears leather like. A formed layer below the surface contributes to the excellent insulating properties and uses of this material. This would apply not only to clothing but also for such items as shoes and boots. This texturing can also be used as one of the tools for achieving visual effects in the artificial food embodiment of this invention.

In U.S. Patent No. 4,588,400, the disclosure of which is hereby incorporated by reference, a microbial cellulose pellicle pad loaded with physiologically-acceptable liquids for medical applications is disclosed. It is part of the instant invention to advance and expand the limited scope of this patent to include cosmetics, soaps, skin-cleaning and hair treating agents. This expansion would also include drugs to be absorbed directly by the skin.

Also, not contemplated by the '400 patent is encapsulating the drug with MC according to a preselected configuration to deliver drugs to a distant part of the digestive system without the drug first having been unduly and prematurely exposed to the digestive liquids of the mouth or stomach.

Microbial cellulose has special usefulness as gums and gels (such as xunthan or algerate), which are well-known classes of materials used for a wide variety of applications. Microbial cellulose pellicles and other variants can be used in any of these known applications. In most instances they will impart superior properties in these environments. One reason is because of their self-supporting properties and characteristics.

Notwithstanding the well-established gum/gel art, microbial cellulose has been found to far exceed the capabilities of agar as a tissue growth medium. This is

because the huge dispersion of submicron hydrophylic cellulose fibers constituting the typical microbial cellulose pellicle is a most superior tissue growing medium for both animal and plant tissues. There are several reasons for this outstanding capability. One is that microbial cellulose gels have exceptional structural strength. Two, they provide a perpetually wet medium for hair roots without drowning them and at the same time ensuring the hair roots have an adequate oxygen supply.

These same characteristics also enable microbial cellulose gels to perform outstandingly as seed coatings. They can enhance and control germination and promote seed development. Moreover, the seeds can be properly spaced in predetermined gel configurations. The gel can be packaged in a container to be dispersed by the user, so that each individual seed can be encapsulated in gel at the user's choice.

Plant tissue can be incorporated into the microbial cellulose gel to obtain effective asexual reproduction from various sources of growable tissues.

The microbial cellulose with seed or tissue or plant suitably incorporated therein can be encased in plastic film containers or tents to obtain a controlled environment. This encompasses moisture control as well as resistance to contaminants, such as viruses and other pathogens. The microbial cellulose can be impregnated with liquid fertilizer, fungicides, and insecticides to further control the environment. The evaporation rate will be particularly controllable, thereby enhancing the longevity of the growing material. Furthermore, if the plastic of the tent is selected to be relatively oxygen permeable, even greater benefits are obtained.

The same type of approach will make these gels useful in fuel cell and battery structure articles, where the electrolyte comprises the liquid phase of the microbial cellulose gel.

Further, a cellulose membrane (pellicle) can be loaded with various cosmetics, skin treating compounds, wrinkle removing compounds and other drugs, emoluments, hair treatments and hormones for the skin. The cellulose membrane containing these ingredients is then placed on the skin. The result is that a thin skin of microbial cellulose forms as an outer layer and prevents evaporation of the ingredient off the skin. This results in a longer and more concentrated exposure of the ingredient on the skin. Similarly, sun screen compositions and mud packs can be enhanced. MC emulsions or suspensions made by physically masticating MC in a mechanical shearing device, such as a blender, can be used per se as a mud or blended with conventional face mud formulations to enhance them. These emulsions and suspensions have a wide variety of other uses as will be apparent from the entirety of this disclosure.

Only one established use of microbial cellulose in pellicle form as a food is known. That is a simple sugar flavored pellicle. The instant invention proceeds considerably beyond that primitive state of the art. Accordingly the present invention incorporates mouth-feel, texture, shape, density and flavor parameters into designing the microbial cellulose pellicle, or dispersion or other gel or physical embodiment to achieve desired effects and result in a particular designed food item.

As one example of this methodology, microbial cellulose, can be obtained and molded or shaped into raw oyster or clam shape/feel pellicles. Clam/oyster flavor can be incorporated into pellicles of requisite texture/shape to obtain very close approximations to the natural material. Under the same focus, steaks and other selected fish, foul and animal food types can be duplicated and artificial sausages, bacon can be formulated. An imitation beef jerky can also be made.

Since cellulose is not digested, microbial produced cellulose provides an ideal non-fattening bulking medium that can be engineered into a wide variety of physical forms and appearances. Accordingly, MC can be a carrier for a wide variety of specialty selected flavors and nutrients for the human or animal body. For instance, it can carry flavors, dyes, fats, lipids, etc. In fact it can be configured to taste and feel like a fat. Puddings, ice creams, salad dressings, creams, spun confections can all be formulated from microcellulose gels and dispersions. Imitation vegetable oils, dressings, mayonnaises, butters, sour creams, cottage cheeses, hard and soft cheeses and spreads are other food variations of viscous food embodiments of this invention.

The types of synthetic foods include:

1) Hard form--this includes artificial potato chips, corn chips, nuts, such as peanuts, macadamia nuts, walnuts, Brazil nuts, and other snacks. Hardness can be accentuated by cross linking and/or prolonging the production of a microbial cellulose to achieve very high microfibril production and density.

2) Soft form--this includes artificial mashed potato, noodles, spaghetti, rice granules, tortillas and other Mexican food products, cake fillings, fudge, candy bar fillings and the like.

3) Fillers--bakery products, artificial flour, cereals, expanded encapsulated microbial cellulose for puffed cereals and artificial popcorn.

4) Ice Nucleation Agents--added and included in various proportions with all frozen foods to control ice formation by nucleation or otherwise to achieve special effects, such as creaminess in popsicles, frozen fruit bars, ice creams, sherbets, and other frozen foods and desserts. Because microbial cellulose interferes with crystalline ice structure, it by itself or

in synergistic admixture with other ice crystal modifiers is particularly useful as a component of liquids to be frozen into smooth texture foods.

Of course, it will be desirable that some of the foods to be formulated will not only be resistant to crystalline ice formation, but also to melting (fudge, butter.) Resistance to both such extremes is a characteristic of microbial cellulose and can be designed and engineered into the products of this invention.

These artificial foods can be made free of deleterious products that sometime plague natural foods, such as virus, bacteria, etc.

The moist stage gel is also useful for testing for the activity of cellulases organisms or substances, especially for the presence of celluloses. In addition, UDPB/cellulose synthase complex on a substrate, such as microbial cellulose gel, could accept glucose or even sucrose to be converted directly into cellulose in vitro.

It is disclosed in copending U.S. Application No. 684,844, and it is apparent that an immense range of chemical derivatives and modifications can be effectuated on fabricated microbial cellulose structures. The invention herein contemplates a considerable, unobviousness stepout from that state of the art.

One concept of this invention is to regard the finely divided in situ submicron-sized cellulose fibers as most ideal starting raw materials for the complete panoply of chemical reactions leading to a wide range of industrial chemical materials. The reactions described in detail in the McGraw Hill Cellulose article for cotton, etc. are considerably improved by utilizing microbial cellulose in a finely divided dispersed state. Thus, an important feature of this invention is to utilize finely divided microbial cellulose fibers obtained either by mechanical dispersion or by agitation during growth of the

microbial cellulose so that chemical reactions can occur therewith in situ. A wide variety of industrial chemicals are made much more efficiently with this approach, since expensive process steps needed to prepare ordinary cellulosic materials for chemical reactions are eliminated.

MC gels, modified with certain grafted side chains, such as PAN, have enormous water-absorbing capacity. Both these and the unmodified versions can be used in agriculture, drilling muds and as thixotropic components of compositions, such as those used for enhanced underground pumping for oil recovery.

Another subclass of the invention comprises process modifications, immediately before and during the microbial-produced cellulose microfibrils production stage and immediately thereafter. These modifications are primarily designed to impact the use properties of the microbial-produced cellulose microfibrils, rather than to improve the economics and efficiency of the process, although in some instances both goals will be obtained.

It is known that ambient, atmospheric oxygen is adequate to obtain reasonable yields of MC. Nevertheless, the efficiency of the oxygen uptake by cellulose producing microbes can be improved. One approach is to increase the concentration of oxygen available to the microbes. This can be accomplished by increasing the volume percent in the ambient gas environment. The other is to dissolve oxygen in the nutrient sodium by physical means such as bubbling or agitation (can form discrete modules of MC) or by chemical compounds such as peroxides. This includes the use of chemical substances such as alcohols from which the microbes can obtain their oxygen needs from the food supply. The pressure of the ambient oxygen can also be increased with hyperbaric techniques.

It is known from previous work that the properties of microbial cellulose can be substantially

modified by the in situ treatment with carboxmethylcellulose(CMC). The effect of CMC is thought to be that of causing and maintaining splaying of micro-fibril ribbon assemblages. In this invention it has been discovered that other materials can substitute for CMC. One such material is polyethylene glycol (PEG), which, in addition to affecting splaying, also beneficially effects strength, flexibility, water, water absorption, optical clarity and the like.

Ribbon splaying can also be accomplished by including certain enzymes active to cellulose, such as cellulase, in the growth medium. Thereafter, once satisfactory splaying has been accomplished, CMC, PEG, etc. can be added as a post reaction step to maintain the splayed state.

The pellicle can also be subject to modification by post-formation viscosity or friction reducing modifying agents. For example, drying a cellulose membrane in the presence of glycerol results in a paper-like product of greatly reduced brittleness and which is exceptionally flexible.

It is also a feature of the invention that different species of bacteria capable of producing a wide variation of cellulose types will be systematically selected to make the cellulose of choice by direct synthesis. If existing strains of bacteria are unable to produce a cellulose of a desired character, the strain can be mutated by genetic or environmental techniques. Thus, Acetobacter has been modified to produce cellulose acetate directly. Other modifications leading to the biosynthesis carboxmethylcellulose and other cellulose derivatives are feasible.

Blends of MC with ordinary plastics will be particularly important in applications requiring a high degree of biodegradeability. An illustrative example is provided U.S. Senate S. 1986 providing that plastic six-pack yokes be degradable.

Capillary electrophoresis is highly promising for rapid and accurate separations of ionic species, i.e. amino acids, peptides, nucleic acids and the like. Many capillary electrophoresis separations based on molecular charge, such as isoelectric focusing, isotachophoresis and micellar electrophoresis are useful. These rely on gel-filled capillaries. The MC gels of this invention are exceptionally well suited for this application, especially since the gels can be prepared in situ in the capillary, if that is appropriate for the separation need.

The filter/membrane/film form and separation capabilities of the MC are especially useful in vapor phase because of the extraordinary surface area of the microfibrils. As an example, cigarette filters made of MC are especially effective at removing adverse components of tobacco smoke. MC can also be used on a much larger scale as a filter to remove or control the level of tobacco smoke in closed areas.

When pellicles or gels of MC are loaded with nutrients, fertilizers, pesticides, fungicides, etc., they can be very effective as long-term, slow release, non-quick-drying units to be placed in areas of highest effectiveness.

Gels, creams and pastes of a wide variety of consistencies can be made and used from MC. All of these, particularly when exposed to heat and cold, have a much higher gel strength and integrity than standard for such items. Industrial pastes and gels can be formulated as rust removers, metal cleaners, foods, condiments, pastes, and the like. Moreover, in addition to the cosmetic-emolument and skin conditioners described above, MC is an ideal hair conditioner and strengthener, because the submicron fibrils can be associated with hair strongly enough and in quantities high enough to have an outstanding beneficial effect on hair appearance, body and feel.

MC is a pure natural cosmetic. Many synthetic conditioners contain chemical residues, such as benzene. This makes them undesirable and in some instances unduly toxic for cosmetic and other uses. Further MC is exceptionally hypoallergenic (not allergenic).

Liposomes containing exceptionally small MC fibrils as drug carriers can be injected into the body. Ordinarily, liposomes are used to inject highly fatty materials into the body that are often incompatible with the body. The liposome compositions contemplated by this invention can carry the submicron MC in the aqueous phase.

Synthetic sweeteners can be sorbed by MC which can then be used as a carrier for a wide variety of frozen and other foodstuffs.

A very high quality cellulose is sold commercially as Microcrystalline Cellulose under the trademark/trade name of AVICEL. This is made from a very finely masticated high purity cellulose paper, such as filter paper. The material is used as a component of foods. It is not very soluble. Comparable physical forms made from MC will be less expensive and superior in almost every significant commercial property.

The present invention will now be further illustrated by certain examples and references which are provided for purposes of illustration only and are not intended to limit the present invention.

Example 1

A Food Delicacy Made from Microbial Cellulose

MC (strain AY 201) was placed into standing culture in a shallow 1 inch tray. The growth medium was Schramm Hestrin Medium. The culture conditions were 28 C, and after 2 weeks, a prominent gel-like membrane

(pellicle) appeared in and completely filled the culture medium of the shallow culture tray. The pellicle was removed, washed with distilled water, then cut into small cubes about 1/2 inches square. The cubes were further soaked in distilled water, then autoclaved at 240 C at 20 p.s.i. for 30 minutes, then rinsed with sterile distilled water. Then the cubes were placed into an aqueous solution saturated with ordinary table sugar (sucrose). The cubes and sugar solution were then autoclaved again at 240 C at 20 p.s.i. for 20 minutes, cooled, then stored under sterile conditions until use. The sugar cubes of MC were eaten as a delicacy. The mouth feel and sweet taste imparted an excellent food delicacy.

Example 2

Sub-Micron Thin Cellulose Films

Strain NQ-5 of Acetobacter xylinum was innoculated into Roux bottles containing 100 ml of Schramm Hestrin Medium and cultured for 3 days at 28 C. At the end of the second day, a thin, transparent pellicle formed at the gas/liquid interface. This pellicle was harvested and cleaned as follows: It was first soaked in distilled water for 3 hours (3 changes), then in detergent (Alconox) for 12 or more hours. Then the pellicle membrane was thoroughly rinsed in distilled water to remove any residual of detergent. The pellicle was then stretched of the lip of a 250 mm glass beaker and allowed to air dry. The resultant membrane was optically clear but exhibited interference colors typical of thin films. The interference colors suggested a dry pellicle membrane thickness of approximately 100 nm. The pellicle membrane is exceedingly strong and has great dimensional stability. When heated to more than 100 C, there were no

apparent changes in the film. In one sample, electron microscopy grids were placed on the pellicle membrane before drying. Upon drying, the thin pellicle membrane was stretched across the EM grids. The pellicle membrane was then directly viewed in the transmission electron microscope. These membranes consist only of one or two layers of cellulose ribbons. The ribbon is the unit of cellulose which emerges from the bacterium and is well known in the literature. If stretching of the wet membrane is isodiametric, the orientation of the ribbons is random. If the wet membrane is stretched in two preferable directions, ordered ribbons are produced.

Some of these sub-micron films were placed in a high vacuum bell jar, and platinum was heated and vaporized onto the surface. The resulting thin films were electo-conductive and had great dimensional stability.

Example 3

Wet Spinning of Microbial Cellulose

A thin pellicle of microbial cellulose was produced as described in Example 2. The cleaned pellicle could be pulled by hand in two directions using a twisting motion and also squeezing. These motions resulted in the formation of a thin strong thread. Such threads could be made as long as 6 inches. These threads can be used like cotton fibers as starting material for yarn and textile production. The advantages of MC threads is their superior mechanical wet and dry strength as well as continuous length of cellulose microfibrils. Threads as small at 50 microns in diameter can be produced by the above technique. During this pulling, the cellulose ribbons co-align into parallel arrays, thus producing a

large number of intermolecular H-bonds between the microfibril clusters. This property results in a superior mechanical strength and dimensional stability of a fiber for weaving.

In another thread making technique, silicon tubing ranging in id diameter from 0.5 mm to 1.00 cm was filled with Schramm and Hestrin Medium innoculated with Acetobacter. After 3 days, microbial cellulose formed within the tubes as exact casts of the silicon molds. The cellulose was then extracted from the molds by pulling, forming a strong cohesive thread. After pulling from the mold, the thread was dried. This type of batch fed or continuous biosyntheseis of threads will be a useful fermentation method for producing high strength fibers for the textile industry.

Example 4

Plant Seed Germination and Seedling Development in Microbial Cellulose

Microbial cellulose pellicles were produced in trays as described in Example 1 above. The pellicles were cleaned with 10% NaOH and detergent (Alconox), autoclaved and rinsed with distilled water. Then the sterile pellicles were inbibed with sterile inorganic nutrient solution for plant seedling development. This solution, known as Bold's Basal Medium, contains inorganic and organic materials to grow photosynthetic organisms. Seeds of radish (Sativis sp) were sterilized in household bleach, rinsed in sterile distilled water, then placed on the surface of the sterile pellicle which was soaked in Bold's Basal Medium. Seedling germination was better than 90%, and the roots did not penetrate the microbial

cellulose substrate, but the root hairs grew into the substrate. Seedling development by this technique is superior to soil or other synthetic substrates since root development into the substrate is hampered. Thus, the young seedlings can be transplanted later into a more disperse and open soil or synthetic substrate for continued growth. Microbial cellulose is an excellent medium to germinate delicate small seeds and spores from ferns, mosses, fungi, etc. Thus it is anticipated to be an excellent substrate for growing mushrooms.

Example 5

Synthetic Leather from Microbial Cellulose

A pellicle was grown as outlined in Example 1 above and cleaned with 10% NaOH and detergent, then rinsed. The pellicle was then subjected to a standardized freeze drying procedure whereby the water content, in the form of ice, is sublimed from the pellicle. This leaves the pellicle ribbons non-collapsed. The feel and strength of this material resembles a fine patent leather good, similar to that of fashionable hand gloves. Preservatives can be added to the never dried material to prevent enzymatic, chemical hydrolysis, or radiation damage to the artificial leather.

Example 6

A Simplified Highly Efficient Fermentor for Microbial Cellulose

A tray similar to that described in Example 1 above was innoculated with Acetobacter. The entire sterile tray was fitted into a previously sterilized

polyethylene bag which is permeable to oxygen. The bag was inflated with air and sealed, then the culture was allowed to synthesize cellulose. The cellulose yield with strain NQ-5 exceeded 35%, and contamination was avoided. The advantages of this system offer sterile environment for bacterial growth and cellulose production in standing culture. To improve the rate of cellulose synthesis, the polyethylene bag itself has been filled with a thin layer of liquid culture medium as described in Example 1, but the bag was inflated with air or oxygen-rich atmosphere so that the liquid surface was not in contact with the bag. The advantages of this fermentor system is increased culture surface area to the oxygen environment (the bottom of the culture vessel was in direct contact with the oxygen-permeable membrane) and the possibility for increasing and controlling the oxygen content of the atmosphere in association with the growing culture. This technique can be modified for batch fed or continuous fermentation of microbial cellulose.

Example 7

A Plasticizer Makes Dried Microbial Cellulose Membranes Less Brittle and Imparts Greater Strength

Microbial cellulose is produced and cleaned as in Example 1 above, but it can be of any shape or form. Before drying, the cleaned pellicle is soaked in a distilled water solution containing 1-3% wt/vol glycerol. After soaking for 24 hours while on a gyratory oscillator to improve penetration, the pellicle is removed and then air dried. The fully dried pellicle has different physical properties from the pellicle dried only from distilled water. The pellicle is very bendable and resists tearing. It also has 50% greater Youngs's Modulus in comparison with air dried clean cellulose.

Example 8

Resin Impregnated Microbial Cellulose

A never dried pellicle produced as described in Example 7 above was dehydrated over a 12 hour period in an ethanol/water series constituting of 25%, 50%, 75% and 100% ethanol. The ethanol soaked pellicle was then subjected to an acetone exchanges consisting of 25%, 50%, 75%, 100%, 100% over a 12 hour period. The pellicle was then infiltrated with a typical electron microscopy resin known as Spurr's resin. The infiltration series was 25% resin and 75% acetone for 3 hours; 50% resin and 50% acetone for 3 hours; 75% resin and 25% acetone for 12 hours; and, 100% resin for 12 hours, followed by a second exchange of 100% resin for 3 hours. The resin/pellice complex was heat polymerized at 65 C for 24 hours. The cellulose ribbon microfibrils imparts a greater strength to the resin. Flexibility is also increased.

Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

What Is Claimed Is:

1. An article of manufacture comprising microfibrils of bacterial cellulose, produced by a process which comprises the following steps:

culturing a cellulose-producing microorganism, capable of reversing its direction during cellulose synthesis, in a nutrient medium comprising an agent which interferes with crystallization, but not polymerization, of said cellulose, wherein said medium is contained in and said culturing occurs in an enclosed plastic container;

withdrawing said cellulose produced from said culture; and

forming said cellulose into an article.

2. The article as claimed in Claim 1, wherein said microorganism is selected from the genera Acetobacter, Rhizobium, Agrobacterium, Pseudomonas, or Alcaligenes.

3. An article as claimed in Claim 2, wherein said microorganism is selected from the genus Acetobacter.

4. An article as claimed in Claim 3, wherein said microorganism is Acetobacter xylinum.

5. An article as claimed in Claim 4, wherein said microorganism in the NQ-5 strain (ATCC 53582) of Acetobacter xylinum.

6. An article as claimed in Claim 1, wherein said agent is selected from glycerol, polyethylene glycol or carboxmethylcellulose.

7. An article as claimed in Claim 6, wherein said agent is carboxymethylcellulose.

8. An article as claimed in Claim 1, comprising the further step of grafting polyacrylonitrile onto said cellulose.

9. An article as claimed in Claim 1, which is formed into a sheet.

10. An article as claimed in Claim 9, wherein said sheet is paper.

11. An article as claimed in Claim 1, which further comprises magnetic material.

12. An article as claimed in Claim 1, which further comprises an electrical material.

13. An article as claimed in Claim 10, wherein said process comprises the further steps of dyeing select cellulose fibers and forming said paper into currency.

14. An article as claimed in Claim 1, which further comprises a thermosetting resin.

15. An article as claimed in Claim 1, wherein said cellulose is formed into a film of a thickness of less than about 0.1 micron.

16. An article as claimed in Claim 1, comprising the further steps of forming said cellulose into a film and vapor depositing an inorganic material onto said cellulose film.

17. An article as claimed in Claim 1, comprising the further steps of forming said cellulose into a film and epitaxially growing an inorganic material on said cellulose.

18. An article as claimed in Claim 1, which is formed into a cloth shape.

19. An article as claimed in Claim 1, wherein said process comprises the further step of freeze-drying said cellulose.

20. An article as claimed in Claim 1, which is formed into a foodstuff.

21. A process for producing an article of manufacture from bacterial cellulose, comprising the steps of:

culturing a cellulose-producing microorganism, capable of reversing its direction during cellulose synthesis, in a nutrient medium comprising an agent which interferes with crystallization, but not polymerization, of said cellulose, wherein said medium is contained in and said culturing occurs in an enclosed plastic container;

withdrawing said cellulose produced from said culture; and

forming said cellulose into an article.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02355

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC
IPC (4): C 12 P 19/04; C 12 R 1/01, 1/02, 1/05, 1/38, 1/41
U. S. Cl: 435/101,822,823,829,874,878; 536/30,56

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U. S.	435/101,822,823,829,874,878; 536/30,56

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,378,431 (Brown, Jr.) 29 March 1982 See the entire document.	1-12 and 14-21
Y, P	US, A, 4,788,146 (Ring et al.) 29 Nov. 1988 See the entire document.	1-12 and 14-21
X	Dillingham et al. (1961), Bacterial Proceedings, Abstract No. A68 See the entire document.	1 and 3-4
Y	Dillingham et al. (1961), Bacterial Proceedings, Abstract No. A68 See the entire document.	1-4,6-7 and 17-18
Y	US, A, 4,400,466 (Azoulay) 23 August 1983 See abstract and columns 1-6.	1-4
Y	US, A, 4,692,408 (Banks et al.) 08 Sept.1987 See abstract and columns 1-6.	1-4
Y	US, A, 4,352,882 (Maury) 05 October 1982 See the entire document.	1-4

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 September 1989	24 OCT 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Pamela S. Webber Pamela S. Webber

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	GB, B, 2,065,688 (Griffith et al.) 05 October 1982 See the entire document.	1-4
Y	US, A, 4,745,058 (Townsley) 17 May 1988 See the entire document.	6 and 7
Y	US, A, 4,416,93 (McKeown) 22 Nov. 1983 See abstract and columns 1-4.	6 and 7
Y	JOURNAL OF APPLIED POLYMER SCIENCE: APPLIED POLYMER SYMPOSIUM, vol. 37,1983, pages 33-78,Brown, Jr. et. al. See the entire document.	1-21

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2.☐ Claim numbers........, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3.☐ Claim numbers___________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	"Cellulose chemistry and its applications," Editors T. P. Nevell et al., 1985, Ellis Horwood Ltd, New York, US; See entire document.	1-21
Y,L	SCIENCE, vol. 218, 1982, The American Association for the Advancement of Science; R. Malcolm Brown, Jr. et al.: "The Experimental Induction of Altered Nonmicrofibrillar Cellulose"	1-21
Y	JOURNAL OF CELL BIOLOGY, vol. 94, 1982, pages 64-69, The Rockefeller University Press; Haigler et al.: "Alteration of In Vivo Cellulose Ribbon Assembly by Carboxymethlcellulose and Other Cellulose Derivatives.	1-21
A	INTERNATIONAL SYMPOSIUM ON WOOD AND PULPING CHEMISTRY, June 9-12, 1981; vol. 3, R. Malcolm Brown, Jr. "Integration of Biochemical and Visual Approaches to the Study of Cellulose Biosynthesis and Degradation."	1-21